THE RELATION BETWEEN KIND OF FOOD, GROWTH, AND STRUCTURE IN AMOEBA[1]

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It is well known that amoebae usually feed on living organisms and that they ordinarily ingest several different kinds. It has, however, been demonstrated that for some species, one kind suffices for growth (Oehler, 1916, 1924; Rice, 1935; Hopkins, 1937). No observations have been made on the relation between the kind of food and the structure of amoebae. This is the main problem involved in the following experiments.

Amoeba proteus and *Amoeba dubia* grown in Hahnert solution[2] containing rice grains were fed on *Chilomonas paramecium* raised on sterile acetate-ammonium[3] and glucose-peptone[4] solutions respectively and *Colpidium striatum* raised on sterile tryptone-phosphate[5] solution. The experiments were made as follows:

Numerous amoebae were taken from vigorous cultures, passed through several separate portions of distilled water so as to remove the food, and then left in distilled water several hours. In this, many of them became stellate in form. The largest of these were selected and five of them put into each of four 6 cc. glass salt dishes containing 3 cc. Hahnert solution each. Then numerous chilomonads or colpidia which by means of the centrifuge had been passed successively through 4 separate portions of fresh Hahnert solutions were added to the solution in each, and left two hours, i.e. until the amoebae had ingested many chilomonads or colpidia, then the amoebae with as little solution as possible were transferred to clean salt dishes containing Hahnert solution. This was repeated until the solution was free of chilomonads or colpidia, after which the process of feeding and transferring was repeated and the number of amoebae in each dish recorded daily for 9 days, then 5

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[2] Hahnert solution—KCl, 4 mg.; $CaCl_2$, 4 mg.; $CaH_4(PO_4)_2$, 2 mg.; $Mg_3(PO_4)_2$, 2 mg.; $Ca_3(PO_4)_2$, 2 mg.; water, 1000 cc.

[3] Acetate-ammonium solution—$NaC_2H_3O_2$, 150 mg.; NH_4Cl, 46 mg.; $(NH_4)_2SO_4$, 10 mg.; K_2HPO_4, 20 mg.; $MgCl_2$, 1 mg.; $CaCl_2$, 1.16 mg.; water, 100 cc.

[4] Glucose-peptone solution—peptone, 8 g.; glucose, 2 g.; water, 1000 cc.

[5] Tryptone-phosphate solution—tryptone, 15 g.; KH_2PO_4, 2 g.; water, 1000 cc. 1000 cc.

of the specimens in each dish were transferred to clean dishes containing Hahnert solution and the rest discarded or used for the study of structure, after which the process of feeding, transferring, and recording

TABLE I

Growth of Amoeba fed on chilomonads and colpidia respectively. Temperature, 21°–25° C.; *x*, all but five discarded; * Several specimens removed for study of structure.

Food	*Amoeba proteus*											
	Number of Specimens											
	August										September	
Chilomonas in glucose-peptone solution	7	10	12	14	16	16	20	23	26	29	1	4
	5	8	8	9	6x	5	6	13	12	11	12*	
	5	9	12	15	11x	5	6	7	7	10	9*	
	5	7	6	7	6x	5	5	7	7	10	9*	
	5	6	7	4	3x	3	0					
Chilomonas in acetate-ammonium solution								5	3	0		
								5	7	0		
								5	7	1		
Colpidium	5	16	24	34	35x	5	12*	17	26	29	42*	7
	3	6	16	23	16x	5	8	13	14	20	23*	24
	4	12	24	50	90x	5	8	9	5	11	15	20
	5	8	17	27	45x	5	7	7	11	18	29	40
	Amoeba dubia											
Chilomonas in glucose-peptone solution	5	6	4	2				5	5	6		
	5	4	3	5				5	5	0		
	5	7	5	4				5	6	3		
	5	4	5	5				5	5	0		
Chilomonas in acetate-ammonium solution								5	2	0		
								5	3	0		
								5	5	2		
Colpidium	5	10	13	17	22x	5	5	5	2	0		
	5	13	24	26	44x	5	3	6	2	1		
	5	9	11	13	21x	5	6	3	3	0		
	5	12	13	12	20x	5	1	0				

was again repeated daily for 16 days. The results obtained are presented in Table I.

This table shows that both *Amoeba proteus* and *Amoeba dubia* fed exclusively either on chilomonads or colpidia increased in number, but

that the increase continued thruout the experiment only in *Amoeba proteus* fed on colpidia. It shows that some of the specimens of *Amoeba proteus* fed on chilomonads were still alive at the close of the experiment, but that the number had decreased; and microscopic examination showed that they were in very poor condition. The table shows that the specimens of *Amoeba dubia* fed on colpidia increased in number much more rapidly and lived much longer than those fed on chilomonads and it shows that for several days those fed on colpidia increased in number as rapidly as *Amoeba proteus* fed on these organisms, but that they then decreased rapidly in number and soon died. The table shows that no increase in number occurred in the specimens of either of the two species of *Amoeba* fed on chilomonads grown in acetate-ammonium solution and that they did not live so long as those fed on chilomonads grown in glucose-peptone solution.

This experiment was repeated in part several times. In some of the tests made, the colpidia used were taken from a culture which contained an unidentified mold, but no bacteria. In some of these tests, the amoebae were left with the food 2 hours, i.e. the same length of time as in the preceding experiments, but in others they were left only 15 minutes and in still others they were left 24 hours.

In the tests in which the amoebae were left with the food only 15 minutes there was no increase in number, either in those fed on chilomonads or in those fed on colpidia. The time was obviously not long enough for the amoebae to ingest sufficient food for growth. The results obtained in the tests in which the amoebae were left with the food 2 and 24 hours respectively are essentially the same as those presented in Table I. That is, in the tests in which chilomonads were used as food, the amoebae usually increased in number fairly rapidly for several days and then decreased, and in those in which colpidia were used, the increase in number continued longer and, under some conditions, doubtless would have continued indefinitely if the tests had not been closed. For example, in one test with *Amoeba proteus* fed on chilomonads, the number increased from 5 to 330 in ten days after which there was a slight increase for a few days, then a gradual decrease to zero, and in another with *Amoeba proteus* fed on colpidia there was a slow, but consistent increase in number for 34 days, i.e. thruout the entire experiment, with no indication of deterioration whatever, altho the increase during this entire time was only from 10 to 106.

The results obtained seem to demonstrate therefore that *Amoeba proteus* can grow and live indefinitely on sterile colpidia as food, but that *Amoeba dubia* cannot, and that neither can live indefinitely on chilomonads as food, but that chilomonads grown in glucose-peptone solution

are more nearly adequate as food than those grown in acetate-ammonium solution.

The chilomonads grown in acetate-ammonium solution contained much starch and little fat, while those grown in glucose-peptone solution contained considerable starch but no fat and they were much smaller than the former (Fig. 1). The difference in their food value is, therefore, doubtless due to difference in their chemical structure and content correlated with the chemical composition of the medium in which they grow. Growth in *Amoeba* is consequently not only correlated with the kind and the quantity of organism they ingest, but also with the physiological condition of the organism ingested.

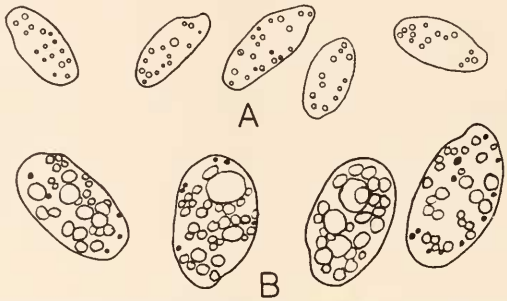

FIG. 1. Camera outlines of *Chilomonas paramecium* showing the effect of the kind of food in the culture medium on size and content. *A*, specimens taken at random from a vigorous culture in sterile glucose-peptone solution; *B*, specimens taken at random from a vigorous culture in acetate-ammonium solution; ○, starch; ●, fat. The flagella are not represented.

Note that the chilomonads grown in glucose-peptone solution were much smaller and contained much less starch and fat than those grown in acetate-ammonium solution. Growth is more rapid in the former solution than in the latter.

In the experiments on growth in *Amoeba* fed exclusively on sterile chilomonads and colpidia respectively, specimens were taken from the cultures at different times and studied in reference to behavior and structure. The results obtained are summarized in the following pages.

The specimens of *Amoeba proteus* which had fed exclusively on colpidia for several days were extraordinarily large (Fig. 2) and literally packed full of globules of fat, especially those which had fed on colpidia from the culture which contained mold.[6] They had only a few

[6] This mold contained much fatty acid but no neutral fat and the colpidia contained enormous quantities of neutral fat but no fatty acid. In fresh cultures the colpidia multiplied rapidly and became abundant in 24 hours. At this time the solution was perfectly clear and the colpidia in it contained but little or no fat. Then the solution gradually became turbid and in 4 or 5 days, mold was clearly visible and at this time the colpidia were well filled with globules of fat and each one usually contained 3 or 4 fragments of mold hyphae or spores which contained liberal quantities of fatty acid which was doubtless changed to neutral fat in the cytoplasm of the colpidia.

pseudopods and these were very short, thick, and blunt without a hyaline cap. They were only slightly attached to the substratum and moved about very slowly and irregularly, now in one direction, then in another, giving the impression of very sluggish, aimless, rolling about. Many had two nuclei. The alpha and beta granules were normal in number and structure, but the bipyramidal crystals were scarce and much

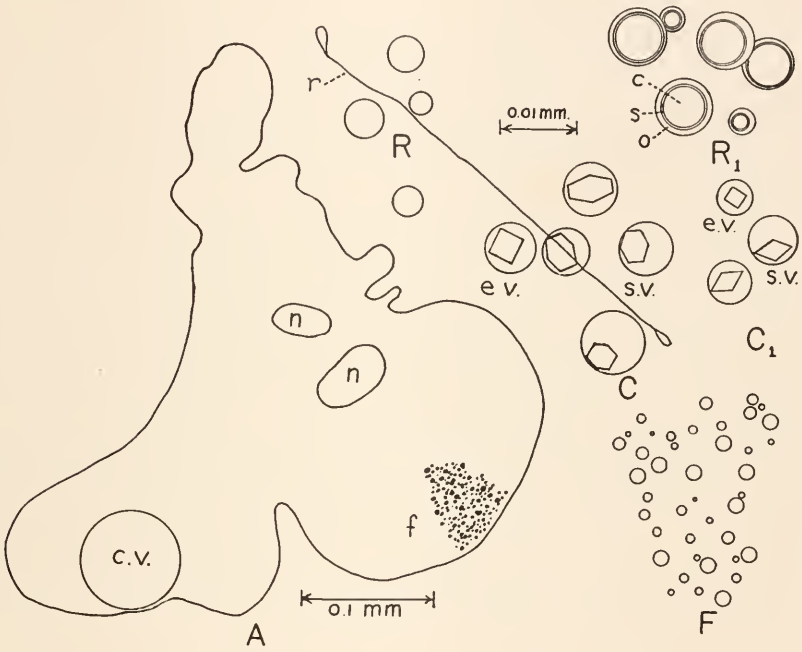

FIG. 2. Camera outlines showing the size, form, and structure of *Amoeba proteus* fed exclusively on colpidia and chilomonads respectively.

A, optical section of *Amoeba proteus* fed on colpidia; *n*, nucleus; *c.v.*, contractile vacuole; *f*, fat globules in one focal plane; *F*, same, enlarged; *C*, bipyramidal crystals in *A*; *R*, largest refractive bodies in *A* (substance in them not differentiated); *r*, one of these drawn out in the form of a fiber; C_1, bipyramidal crystals in a specimen fed on chilomonads; *s.v.*, side view; *e.v.*, end view; R_1, refractive bodies in a small area in an optical plane in a specimen fed on chilomonads (substance in these highly differentiated); *o*, outer layer; *s*, shell; *c*, central substance; *mm*, projected scale.

shorter and thicker and more truncated than usual and there were usually only a few spherical bodies and some specimens had none at all. The spherical bodies were with few exceptions very small and the substance in them undifferentiated. There was nothing in them similar to the fragile shell usually found and all the substances in them usually stained crimson with neutral red, but the central portion often appeared lighter

in color and somewhat more granular than the rest, and did not stain so readily. This substance was usually so elastic that if the bodies were released after they had been flattened by means of pressure on the cover-glass they soon assumed their original shape and it was so adhesive and viscous that if the cover-glass was pushed sidewise on the slide after the bodies had been flattened by pressure on it, the substance in them, owing to adhesions to the glass, was often drawn out in the form of a long slender fiber (Fig. 2).

It is consequently obvious that if the food of specimens of *Amoeba proteus* is restricted to colpidia great changes occur in them in reference to size, form, behavior, and structure; in fact, changes so great that if such specimens were examined without information as to their origin they would certainly be designated as a new species and probably as a new genus.

Specimens of *Amoeba proteus* which for several days had fed exclusively on chilomonads were normal in size, form, and activity; but they contained an extraordinarily large number of spherical bodies (often a thousand or more) and numerous bipyramidal crystals and very little or no fat. The spherical bodies were relatively very large and the substances in them well differentiated into a central mass surrounded with a prominent fragile shell which was covered with a thin layer of oily substance (Fig. 2). In solutions containing neutral red, the outer layer became deep red (crimson) in color, but the central portion and the shell did not stain. The spherical bodies in these amoebae were, therefore, similar to some described by Mast and Doyle (1935, p. 167) but differed radically in number, size, and structure from those found in the amoebae fed exclusively on colpidia.

The bipyramidal crystals were relatively long and but little truncated and in some specimens as many as 2 percent of them were not truncated at all (Fig. 2).

The facts that there were many more refractive bodies in the amoebae which had fed on chilomonads than in those which had fed on colpidia and that they were much larger and the substance in them much more differentiated, show that these structures are closely correlated with the kind of food ingested. They therefore support the contention of Mast and Doyle (1935, p. 291) and others that they are cytoplasmic inclusions and not cytoplasmic structures, i.e. secondary nuclei (Calkins, 1905), cysts (Taylor, 1924), Golgi bodies (Brown, 1930), mitochondria, (Horning, 1925, 1928), vacuome (Volkonsky, 1933).

Amoeba dubia usually contains relatively few crystals (some irregular or roughly bipyramidal in form with the edges and corners rounded, and some thin rectangular plate-like in form) and not much fat.

In the specimens fed on colpidia the irregular crystals decreased greatly in number and often disappeared entirely and the plate-like crystals increased considerably and there was marked accumulation of fat, altho not nearly so much as in *Amoeba proteus*. In those fed on chilomonads the irregular crystals increased greatly in number and the plate-like crystals decreased considerably and the fat usually disappeared.

There was no significant change in size, form, or activity in those fed on colpidia or those fed on chilomonads.

Summary

1. If specimens of *Amoeba proteus* are fed exclusively on colpidia, they become very large and extremely fat and sluggish and grow and multiply slowly, but indefinitely. The refractive bodies in them decrease greatly in number and size and their content becomes homogeneous and very adhesive, elastic and viscous. The crystals decrease in number and become shorter and more truncated.

2. If they are fed exclusively on chilomonads, they grow and multiply for several days, then decrease in number and soon die, but they live longer if the chilomonads have grown in glucose-peptone solution than if they have grown in acetate-ammonium solution. The refractive bodies increase greatly in size and number and the content of these bodies becomes sharply differentiated; the bipyramidal crystals increase in number and become less truncated, and the fat decreases in quantity.

3. If specimens of *Amoeba dubia* feed exclusively on chilomonads, they multiply for a few days, then cease and soon die. The irregular-shaped crystals increase and the plate-like crystals decrease considerably in number and the fat disappears.

4. If they feed exclusively on colpidia, they multiply more and live longer than if they feed exclusively on chilomonads, but they do not live indefinitely. The plate-like crystals increase in number and the irregularly shaped crystals usually disappear entirely and the fat increases in quantity, but not so much as it does in *Amoeba proteus*.

5. *Amoeba* is in reference to form, size, behavior, and structure closely correlated with the kind of organisms it eats and their physiological condition.

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